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Effect of intestinal function-recovering decoction on treatment of multiple organ dysfunction syndrome in rats

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ABSTRACT

Objective: To analyze the effect of intestinal function-recovering decoction on multiple organ dysfunction syndrome in rats, and to investigate a novel solution to multiple organ dysfunction syndrome. **Methods:** Multiple organ dysfunction syndrome was induced in 60 Sprague-Dawley rats by intestinal ischemia-reperfusion combined with cecal ligation and puncture. Then these rats were intragastrically administered physiological saline (group I, n=20), ampicillin (group II, n=20) or intestinal function-recovering decoction (group III, n=20). After treatment, serum malondialdehyde and superoxide dismutase levels were compared among three groups. Simultaneously, bacterial culture of various organ tissues was performed and bacterial and endotoxin translocation were observed. **Results:** Compared with group I, serum malondialdehyde, alanine aminotransferase and aspartate aminotransferase levels were significantly decreased (all P<0.05) and serum superoxide dismutase level was significantly increased (P<0.05) in the group III. However, there were no significant differences in these indices between groups II and III (P>0.05). The rate of bacterial translocation in the groups II and III was significantly lower than in the group I (P<0.05), and no significant difference was observed between groups II and III (P>0.05). **Conclusions:** Intestinal function-recovering decoction can significantly reduce endotoxin and bacterial translocation and stabilize enteral oxidative-antioxidative balance.

1. Introduction

Multiple organ dysfunction syndrome (MODS) is a common critical illness in Intensive Care Unit patients. The intestine plays an important role in maintaining organism's nutrient and physiological functions, participates in posttraumatic stress reaction, and is a "starter" of MODS[1]. It has been demonstrated that the purgative therapy shows satisfactory therapeutic effects on MODS[2]. Clinical studies and animal experiments have shown that intestinal function-recovering decoction can reduce endotoxin and bacterial translocation, protect intestinal mucous membrane, decrease serum endotoxin level and alleviate systematic inflammatory reaction[3]. In this study, we established Sprague-Dawley rat models of MODS and intragastrically administered physiological saline, ampicillin or intestinal function-recovering decoction to investigate the effect of intestinal function-recovering decoction on serum malondialdehyde (MDA) and superoxide dismutase (SOD) levels and bacterial and endotoxin translocation.

2. Materials and methods

2.1. Animals

Sixty Sprague-Dawley rats of clean grade and either gender, weighing (241.3±16.5) g, were provided by

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Laboratory Animal Center, First Hospital, Jilin University, China and included in this study. All experimental procedures were in strict accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, issued by the Ministry of Science and Technology of China.

2.2. Main instruments and reagents

OLYPAS optical microscope, T6 UV spectrophotometer, ultracentrifuge, MDA kit and SOD kit were provided by Nanjing Jiancheng Bioengineering Institute, Nanjing, China. Ampicillin was provided by Shenzhen Haibin Pharmaceutical Co., Ltd., Shenzhen, China. Intestinal function-recovering decoction was prepared by Pharmaceutical Product Center, First Hospital, Jilin University, China. This decoction was composed of Baizhu (*Largehead atractylodes* Rhizome, Rhizoma *Atractylodis macrocephalae*) 15 g, Chenpi (Dried tangerine peel, *Pericarpium citri* Reticulatae) 15 g, Chishao (Red peony poot, Radix *Paeoniae rubra*) 15 g, Huomaren (Hemp seed, Semen cannabis) 30 g, Muxiang (Common Aucklandia root, Radix Aucklandiae) 12 g, Taoren (Peach seed, Semen persicae) 12 g, Zhishi (Immature orange fruit, Fructus *Aurantii immaturus*) 12 g, Danshen (Danshen root, Radix *Salviae miltiorrhiae*) 12 g, Dangshen (Tangshen, Radix Codonopsis) 15 g, and Dahuang (Rhubarb, Radix et Rhizoma Rhei) 20 g (added lastly).

2.3. Establishment of MODS models in rats

Rat models of MODS were established by intestinal ischemia-reperfusion combined with cecal ligation and puncture[4]. After fasting for 24 h, MODS rats were anesthetized by intraperitoneal injection of chloral hydrate and placed in a dorsal position. Following shaving and disinfection, a median abdominal incision was made to expose the superior mesenteric artery. Then the root of the superior mesenteric artery was occluded for 45 min and then re-perfused. During artery occlusion, isotonic saline solution was intraperitoneally injected intermittently to prevent transient hypovolemia after loosening the bulldog clamp. The ileocecal artery was dissociated and then cecal ligation inside the arch of aorta was performed. A pore with 2 mm diameter was punctured at the free end of the cecum once the ileocolon was determined patent. Then the abdominal cavity was closed.

2.4. Methods

All 60 rat models of MODS were intragastrically administered 10 mL/kg physiological saline (group Ⅰ, n=20); 89.25 mg/kg ampicillin (group Ⅱ, n=20) or 10 mL/kg intestinal function-recovering decoction (group Ⅲ, n=20). Each administration was repeated twice per day for 2 successive days.

2.5. Indices observation

At 48 h after successful induction of MODS, under anesthesia by chloral hydrate, portal venous and peripheral venous blood samples were obtained from each group rats and centrifuged at low temperature. The supernatant was collected and serum endotoxin level was quantitatively determined by limulus amebocyte lysate test. Ileal content sample was taken for determination of endotoxin level. MDA level was determined by thiobarbituric acid method and SOD level by xanthine oxidase method. Rat mesenteric lymph node, liver, kidney, lung and spleen tissue samples were harvested from each group, prepared into homogenates and then inoculated into Petri dishes. Superior mesenteric vein blood sample was taken and cultured in culture medium at 37 ℃ for 48 h for bacterial culture. If the number of colonies was greater than 100 colonies/g, then bacterial culture-positive was considered. The rate of bacterial translocation was calculated by the number of organs with bacterial culture-positive findings/total number of organs cultured. After laparotomy, ileal tissue sample was harvested 5 cm away from the cecum and stained by hematoxylin-eosin for observing the pathomorphological changes of intestinal mucous membrane.

2.6. Statistical analysis

All experimental data were statistically processed using SPSS12.0 software. Measurement data were expressed as mean±SD and t-test was performed. *Chi*-square test of numeration data was performed. A level of P<0.05 was considered as statistically significance.

3. Results

3.1. Comparison of endotoxin level in rats among three groups

Endotoxin level in the peripheral venous blood, portal venous blood and ileal content in the group Ⅲ was significantly lower than in the group I (P<0.01). There was no significant difference in endotoxin level in the peripheral venous blood, portal venous blood and ileal content between groups Ⅱ and Ⅲ (P > 0.05) (Table 1).

Table 1

Endotoxin level in rats among three groups.

Group	Peripheral venous blood (eu/ mL)	Portal venous blood (eu/mL)	Ileal content (10^6 eu/g)
Ⅰ	0.502±0.040	0.580±0.015	0.303±0.019
Ⅱ	0.484±0.023*	0.562±0.019[a]	0.311±0.017[a]
Ⅲ	0.289±0.015*	0.450±0.018[a]	0.201±0.015[a]

[a]P < 0.05, *vs.* group Ⅰ.

3.2. Comparison of serum MDA and SOD levels among three groups

Compared to group Ⅰ, serum MDA level was significantly lower (P<0.01), and serum SOD level was significantly higher (P<0.01) in the group Ⅲ. There were no significant differences in MDA and SOD levels between groups Ⅱ and Ⅲ (P>0.05; Table 2).

Table 2
Serum MDA and SOD levels among three groups

Group	MDA (mmol/ mL)	SOD (u/ mL)
Ⅰ	7.56±0.25	85.41±6.10
Ⅱ	7.38±0.36[a]	107.40±7.67[a]
Ⅲ	5.59±0.28[a]	133.82±5.39[a]

[a]P < 0.05, *vs.* group Ⅰ.

3.3. Comparison of bacterial translocation among three groups

Bacterial translocation rate was highest in the group Ⅰ (85.3%), followed by group Ⅱ (45.6%) and lastly group Ⅲ (43.87%). The bacterial translocation rate in the group Ⅰ was significantly higher than in the groups Ⅱ and Ⅲ (P<0.05). No significant difference was observed between groups ⅡII and Ⅲ (P>0.05).

3.4. Pathomorphological changes in ileal mucous membrane in groups Ⅰ and Ⅲ

Through the microscopy, the group Ⅰ showed severely damaged ileal mucous membrane epithelial cells, some of which were shed, with disrupted villi and obvious infiltration of inflammatory cells; however, the injuries to the ileal mucous membrane epithelial cells and villi were greatly alleviated in the group Ⅲ (Figure 1).

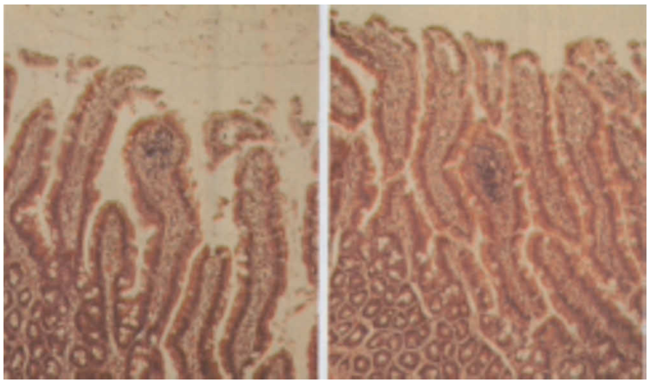

Figure 1. Pathomorphological changes in rat ileal mucous membrane in groups Ⅰ and Ⅲ (×200).

4. Discussion

Three kinds of pathological mechanisms underlying MODS have been clinically accepted: theory of microcirculatory disturbance; theory of inflammatory mediators and cytokines; and theory of endotoxin translocation and intestinal bacteria[5-7]. When the organisms are subjected to severe trauma, a large amount of inflammatory mediators and cytokines release, leading to intestinal bacterial and endotoxin translocation and finally resulting in septicemia and endotoxemia[8]. Therefore, inhibiting bacterial and endotoxin translocation and protecting intestinal mucous membrane barrier and its function are of great significance for prevention and treatment of MODS[9-11].

In the recipe of intestinal function-recovering decoction, Dahuang has purgative activity; Baizhu and Chenpi can nourish the stomach and spleen; Huomaren can loosen bowel to relieve constipation; Dangshen can strengthen the middle warmer and benefit vital energy; Danshen can activate blood to promote menstruation; Zhishi can reduce Qi stagnation, relieve indigestion and abdominal distension and resolve sputum; Muxiang can promote the flow of Qi; Taoren and Chishao can promote blood circulation by removing blood stasis. A combination of these ingredients can promote enterocinesia and thereby contribute to recovery of postoperative intestinal function[8]. Modern pharmacological studies have demonstrated that Huomaren and Baizhu can strength gastrointestinal motility, accelerate bacterial and endotoxin discharge, inhibit bacterial colonization and reduce the production of endotoxin; Dahuang, Muxiang, Danshen and Dangshen possess anti-bacterial effects, inhibit enteral bacterial translocation and protect intestinal mucous membrane. Ampicillin exhibits strong anti-bacterial capacity; nevertheless, while killing bacteria, ampicillin results in the production of endotoxin, destroying intestinal biological barrier, aggravating the damage to the organism caused by bacterial and endotoxin translocation[12-16]. MDA level can reflect the degree of lipid peroxidation *in vivo* and SOD activity can directly reflect the ability of organism to get rid of oxygen free radicals[17-19]. Modern pharmacological studies have demonstrated that Danshen, Dahuang and Taoren exhibit anti-oxidative capacity. In the recipe of intestinal function-recovering decoction, Danshen can get rid of oxygen free radicals, inhibit lipid peroxidation, increase SOD level *in vivo* and alleviate the injury to ischemic tissue caused by reperfusion[20].

Taken together, in this study, obvious bacterial and endotoxin translocation was observed in MODS rat models established by intestinal ischemia-reperfusion combined with cecal ligation and puncture. Endotoxin level and bacterial translocation rate in rats undergoing intragastric administration of intestinal function-recovering decoction were significantly decreased compared to those in rats receiving intragastric administration of physiological saline. These findings suggest that intestinal function-recovering decoction can dilute and physically wash intestinal endotoxin, markedly reduce endotoxin and bacterial translocation and stabilize enteral oxidative-antioxidative balance in rats with MODS.

Conflict of interest statement

We declare that we have no conflict of interest.

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